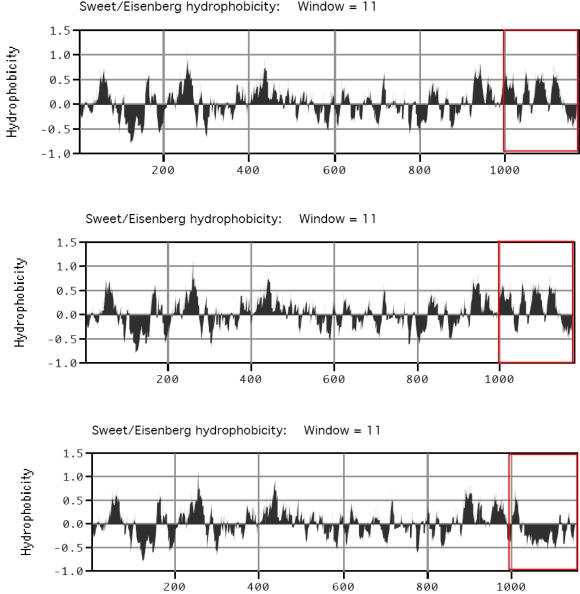
Supplemental Figures and Legends

Figure S1. Sequence alignment of Atp13a2 Isoforms. Accession Numbers: Isoform 1 NP_071372.1, Isoform 2 NP_001135445.1, Isoform 3 NP_001135446.1

ATP13A2.bolom 1 1 M S A D S S P L V G S T P T G Y G T L T I G T S I D P L S S S V S S V R L S G Y C G S P W R V I G Y H V V V W M M A G I P L L L ATP13A2.bolom 2 1 M S A D S S P L V G S T P T G Y G T L T I G T S I D P L S S S V S S V R L S G Y C G S P W R V I G Y H V V V W M M A G I P L L L ATP13A2.bolom 3 1 M S A D S S P L V G S T P T G Y G T L T I G T S I D P L S S V S S V R L S G Y C G S P W R V I G Y H V V V W M M A G I P L L L M S A D S S P L V G S T P T G Y G T L T I G T S I D P L S S V S S V R L S G Y C G S P W R V I G Y H V V V W M M A G I P L L L M S A D S S P L V G S T P T G Y G T L T I G T S I D P L S S V S S V R L S G Y C G S P W R V I G Y H V V V W M M A G I P L L L	FRWKPLWGVRL ⁷⁵
ATP13A2 looform 278 R L R P C N L A H A E T L V I E I R D K E D S S W Q L F T V Q V Q T E A I G E G S L E P S P Q S Q A E D G R S Q A A V G A V P E ATP13A2 looform 378 R L R P C N L A H A E T L V I E I R D K E D S S W Q L F T V Q V Q T E A I G E G S L E P S P Q S Q A E D G R S Q A A V G A V P E	SAWKDTAQLHK SAWKDTAQLHK SAWKDTAQLHK SAWKDTAQLHK SAWKDTAQLHK
ATP13A2 boform 1 151 S E E A V S V G Q K R V L R Y Y L F Q G Q R Y I W I E T Q Q A F Y Q V S L L D H G R S C D D V H R S R H G L S L Q D Q M V R K A ATP13A2 boform 3 151 S E E I A K R V L R Y Y L F Q G Q R Y I W I E T Q Q A F Y Q V S L L D H G R S C D D V H R S R H G L S L Q D Q M V R K A ATP13A2 boform 3 ISI S E E I A K R V L R Y Y L F Q G Q R Y I W I E T Q Q A F Y Q V S L L D H G R S C D D V H R S R H G L S L Q D Q M V R K A ATP13A2 boform 3 ISI S E E I A K R V L R Y Y L F Q G Q R Y I W I E T Q Q A F Y Q V S L L D H G R S C D D V H R S R H G L S L Q D Q M V R K A ATP13A2 boform 4 S E E I A K R V L R Y Y L F Q G Q R Y I W I E T Q Q A F Y Q V S L L D H G R S C D D V H R S R H G L S L Q D Q M V R K A	I Y G P N V I S I P V I Y G P N V I S I P V I Y G P N V I S I P V I Y G P N V I S I P V I Y G P N V I S I P V
A17913A2 boform 1 226 K S Y P Q L L V D E A L N P Y Y G F Q A F S I A L W L A D H Y Y W Y A L C I F L I S S I S I C L S L Y K T R K Q S Q T L R D M V A17913A2 boform 2 221 K S Y P Q L L V D E A L N P Y Y G F Q A F S I A L W L A D H Y Y W Y A L C I F L I S S I S I C L S L Y K T R K Q S Q T L R D M V A17913A2 boform 3 221 K S Y P Q L L V D E A L N P Y Y G F Q A F S I A L W L A D H Y Y W Y A L C I F L I S S I S I C L S L Y K T R K Q S Q T L R D M V A17913A2 boform 3 221 K S Y P Q L L V D E A L N P Y Y G F Q A F S I A L W L A D H Y Y W Y A L C I F L I S S I S I C L S L Y K T R K Q S Q T L R D M V	<pre>< L S M R V C V C R P 295 < L S M R V C V C R P 295</pre>
ATP13A2 boform 1 301 G G E E E W V D S S E L V P G D C L V L P Q E G G L M P C D A A L V A G E C M V N E S S L T G E S I P V L K T A L P E G L G P Y ATP13A2 boform 1 306 G G E E E W V D S S E L V P G D C L V L P Q E G G L M P C D A A L V A G E C M V N E S S L T G E S I P V L K T A L P E G L G P Y ATP13A2 boform 1 206 G G E E E W V D S S E L V P G D C L V L P Q E G G L M P C D A A L V A G E C M V N E S S L T G E S I P V L K T A L P E G L G P Y ATP13A2 boform 3 206 G G E E E W V D S S E L V P G D C L V L P R F G G E E W V D S S E L V P G D C L V L P R E G G L M P C D A A L V A G E C M V N E S S L T G E S I P V L K T A L P E G L G P Y G G E E E W V D S S E L V P G D C L V L P Q E G G L M P C D A A L V A G E C M V N E S S L T G E S I P V L K T A L P E G L G P Y	CAETHRRHTLF CAETHRRHTLF 370 CAETHRRHTLF 370 370 CAETHRRHTLF
ATP13A2 boform 1 376 C G T L I L Q A R A Y V G P H V L A V V T R T G F C T A K G G L V S S I L H P R P I N F K F Y K H S M K F V A A L S V L A L L G ATP13A2 boform 2 371 C G T L I L Q A R A Y V G P H V L A V V T R T G F C T A K G G L V S S I L H P R P I N F K F Y K H S M K F V A A L S V L A L L G ATP13A2 boform 3 371 C G T L I L Q A R A Y V G P H V L A V V T R T G F C T A K G G L V S S I L H P R P I N F K F Y K H S M K F V A A L S V L A L L G ATP13A2 boform 3 371 C G T L I L Q A R A Y V G P H V L A V V T R T G F C T A K G G L V S S I L H P R P I N F K F Y K H S M K F V A A L S V L A L L G C G T L I L Q A R A Y V G P H V L A V V T R T G F C T A K G G L V S S I L H P R P I N F K F Y K H S M K F V A A L S V L A L L G	T I Y S I F I L Y R N T I Y S I F I L Y R N 445 T I Y S I F I L Y R N 445 T I Y S I F I L Y R N
ATP13A2 boform 1 451 R V P L N E I V I R A L D L V T V V V P P A L P A A M T V C T L Y A Q S R L R R Q G I F C I H P L R I N L G G K L Q L V C F D K ATP13A2 boform 2 466 R V P L N E I V I R A L D L V T V V V P P A L P A A M T V C T L Y A Q S R L R R Q G I F C I H P L R I N L G G K L Q L V C F D K ATP13A2 boform 3 466 R V P L N E I V I R A L D L V T V V V P P A L P A A M T V C T L Y A Q S R L R R Q G I F C I H P L R I N L G G K L Q L V C F D K ATP13A2 boform 3 466 R V P L N E I V I R A L D L V T V V V P P A L P A A M T V C T L Y A Q S R L R R Q G I F C I H P L R I N L G G K L Q L V C F D K	FGTLTEDGLDV 520
APP1342 boform 1 506 M G V V P L K G Q A F L P L V P E P R R L P V G P L L R A L A T C H A L S R L Q D T P V G D P M D L K M V E S T G W V L E E E P APP1342 boform 3 521 M G V V P L K G Q A F L P L V P E P R R L P V G P L L R A L A T C H A L S R L Q D T P V G D P M D L K M V E S T G W V L E E E P APP1342 boform 3 M G V V P L K G Q A F L P L V P E P R R L P V G P L L R A L A T C H A L S R L Q D T P V G D P M D L K M V E S T G W V L E E E P M G V V P L K G Q A F L P L V P E P R R L P V G P L L R A L A T C H A L S R L Q D T P V G D P M D L K M V E S T G W V L E E E P M G V V P L K G Q A F L P L V P E P R R L P V G P L L R A L A T C H A L S R L Q D T P V G D P M D L K M V E S T G W V L E E E P	A A D S A F G T Q V L 600 A A D S A F G T Q V L 595 A A D S A F G T Q V L 595 A A D S A F G T Q V L
APP13A2 botom 1 601 A V M R P P L W E P Q L Q A M E E P P V P V S V L H R F P F S A L Q R M S V V V A W P G A T Q P E A Y V K G S P E L V A G L C T APP13A2 botom 2 596 A V M R P P L W E P Q L Q A M E E P P V P V S V L H R F P F S S A L Q R M S V V V A W P G A T Q P E A Y V K G S P E L V A G L C T APP13A2 botom 3 596 A V M R P P L W E P Q L Q A M E E P P V P V S V L H R F P F S S A L Q R M S V V V A W P G A T Q P E A Y V K G S P E L V A G L C T APP13A2 botom 3 596 A V M R P P L W E P Q L Q A M E E P P V P V S V L H R F P F S S A L Q R M S V V V A W P G A T Q P E A Y V K G S P E L V A G L C T APP15A2 botom 3 596 A V M R P P L W E P Q L Q A M E E P P V P V S V L H R F P F S S A L Q R M S V V V A W P G A T Q P E A Y V K G S P E L V A G L C T	IPETVPTDFAQ 670
A7P13A2 toform 1 G7G M L Q S Y T A A G Y R V V A L A S K P L P T V P S L E A A Q L T R D T V E G D L S L L G L L V M R N L L K P Q T T P V I Q A L A7P13A2 toform 2 G7I M L Q S Y T A A G Y R V V A L A S K P L P T V P S L E A A Q Q L T R D T V E G D L S L L G L V M R N L L K P Q T T P V I Q A L A7P13A2 toform 3 G7I M L Q S Y T A A G Y R V V A L A S K P L P T V P S L E A A Q Q L T R D T V E G D L S L L G L V M R N L L K P Q T T P V I Q A L A7P13A2 toform 3 G7I M L Q S Y T A A G Y R V V A L A S K P L P T V P S L E A A Q Q L T R D T V E G D L S L L G L L V M R N L L K P Q T T P V I Q A L	R T R I R A V M V T 745 R T R I R A V M V T 745
A1713A2 totom 1 751 16 D N Q T V T V A P C M H	799
ATPI3A2 bofom 1 826 S G P T F G I I V K H F P K L L P K V L V Q G T V F A R M A P E Q K T E L V C E L Q K L Q Y C V G M C G D G A N D C G A L K A A I ATPI3A2 bofom 2 821 S G P T F G I I V K H F P K L L P K V L V Q G T V F A R M A P E Q K T E L V C E L Q K L Q Y C V G M C G D G A N D C G A L K A A I ATPI3A2 bofom 3 800 S G P T F G I I V K H F P K L L P K V L V Q G T V F A R M A P E Q K T E L V C E L Q K L Q Y C V G M C G D G A N D C G A L K A A I	0 V G I S L S Q A E A 0 V G I S L S Q A E A 0 V G I S L S Q A E A 0 V G I S L S Q A E A 0 V G I S L S Q A E A
A171342 toform 1 901 \$V V S P F T S S M A S I E C V P M V I R E G R C S L D T S F S V F K Y M A L Y S L T Q F I S V L I L Y T I N T N L G D L Q F L J A171342 toform 2 966 \$V V S P F T S S M A S I E C V P M V I R E G R C S L D T S F S V F K Y M A L Y S L T Q F I S V L I L Y T I N T N L G D L Q F L J A171342 toform 3 956 \$V V S P F T S S M A S I E C V P M V I R E G R C S L D T S F S V F K Y M A L Y S L T Q F I S V L I L Y T I N T N L G D L Q F L J A171342 toform 3	A I D L V I T T T V A 970 A I D L V I T T T V A 931
ATP13A2 botom 1 976 {V L M S R T G P A L V L G R V R P P G A L L S V P V L S S L L L Q M V L V T G V Q L G G Y F L T L A Q P W F V P L N R T V A A P 1 ATP13A2 botom 2 971 {V L M S R T G P A L V L G R V R P P G A L L S V P V L S S L L Q M V L V T G V Q L G G Y F L T L A Q P W F V P L N R T V A A P ATP13A2 botom 3 902 {V L M S R T G P A L V L G R V R P P G A L L S V P V L S S L L L Q M V L V T G V Q L G G Y F L T L A Q P W F V P L N R T V A A P V L M S R T G P A L V L G R V R P P G A L L S V P V L S S L L L Q M V L V T G V Q L G G Y F L T L A Q P W F V P L N R T V A A P	DNLPNYENTVV 1045 DNLPNYENTVV 1006
A771342 toform 1 1051 F S L S S F Q Y L I L A A A V S K G A P F R P L Y T N	RNITDTGFKLL1119 RNI <u>TDTGFK</u> LL1114 AAAARRPPE <u>VV</u> 1081 RNITDTGFKLL
ATPI3A2 botom 1 1120 LLG LVTLNFVGAFMLESVLDQCLPACLRRLRPKRASKKR FKQLERELAEQPWPP ATPI3A2 botom 2 1115 LLG LVTLNFVGAFMLESVLDQCLPACLRRLRPKRASKKR FKQLERELAEQPWPP ATPI3A2 botom 3 108 QLGVUDVCV + FKQLERELAEQPWPP ATPI3A2 botom 3 108 QLGVUDVCV + FKQLERELAEQPWPP ATPI3A2 botom 3 108 QLGVUDVCV + FKQLERELAEQPWPP FKQLERELAEQPWPP	I P A G P I 1174
ATP13A2 boform 1 1180 R 1180 ATP13A2 boform 2 1175 R 1175 ATP13A2 boform 3 1177 S V 1158	

Figure S2. Atp13a2 Hydrophobicity Profiles. (A) Isoform-1. (B) Isoform-2. (C) Isoform-3. The Cterminal region of each isoform is outlined in red. Note Isoform-3 is missing the last two transmembrane domains (eight total) compared to isoforms 1 and 2 (ten total).



Sweet/Eisenberg hydrophobicity: Window = 11

Α

Β

С

Figure S3. Predicted Atp13a2^{Isoform 1} Topology and Mutation Sites. Diagram depicts conserved P-type ATPase motifs (colored ovals) and mutation events (colored stars). The conserved domains are as follows: actuator domain (A-domain), the phosphorylation domain (P-domain) and the nucleotide binding domain (N-domain). The P-domain contains the conserved aspartic acid residue which is phosphorylated during the catalytic cycle. The mutation events are as follows: Ex13, an in-frame deletion of exon13 which removes 111 nucleotides and a portion of the third transmembrane domain; Dup22, a duplication of 22 base pairs in exon16 which creates a frameshift and deletes the last 6 transmembrane domains; ΔC , a deletion of cytosine 3057 which results in a frameshift and the removal of the last three transmembrane domains.

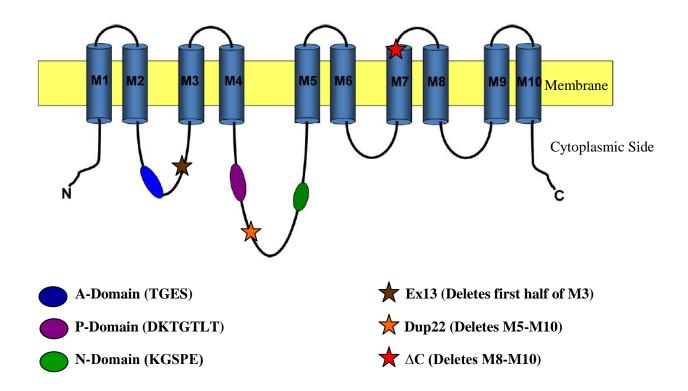
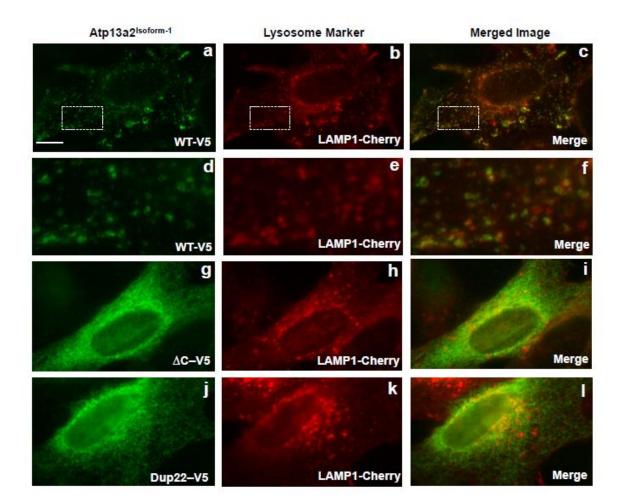
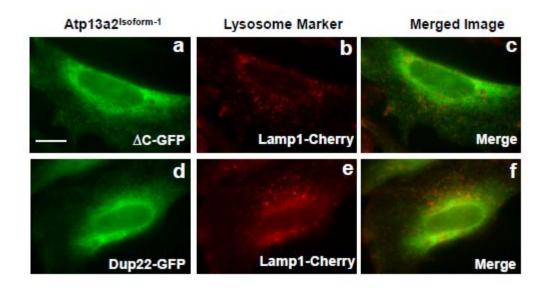


Figure S4. Atp13a2 Localization. (A) HeLa cells were cotransfected with LAMP1-Cherry and V5-tagged wild type (a-f), ΔC (g-i) or Dup22 (j-l) Atp13a2^{lsoform-1} and visualized using fluorescence microscopy. (d-f) Magnification of region outlined in panels (a-c). (B) HeLa cells were cotransfected with LAMP1-Cherry and GFP-tagged ΔC (a-c) or Dup22 (d-f) Atp13a2^{lsoform-1} and visualized using fluorescence microscopy. (C) HeLa cells were cotransfected with LAMP1-Cherry and GFP-tagged wild type (a-c), ΔC (d-f) or Ex13 (g-i) Atp13a2^{lsoform-3} and visualized using fluorescence microscopy. Bar, 5µm

Α





С

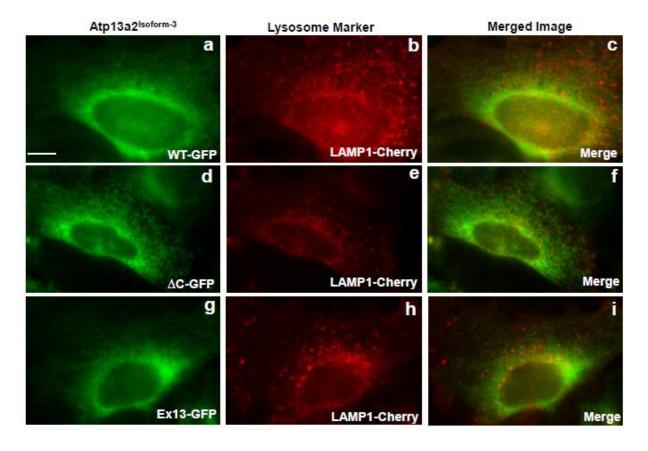


Figure S5. Atp13a2^{Isoform-1} Glycosylation. HeLa cells stably expressing V5-tagged wild type ATP13A2^{Isoform-1} were treated with tunicamycin for the indicated time periods and analyzed by immunoblotting.

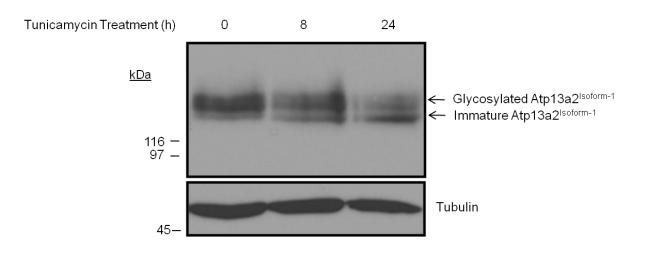
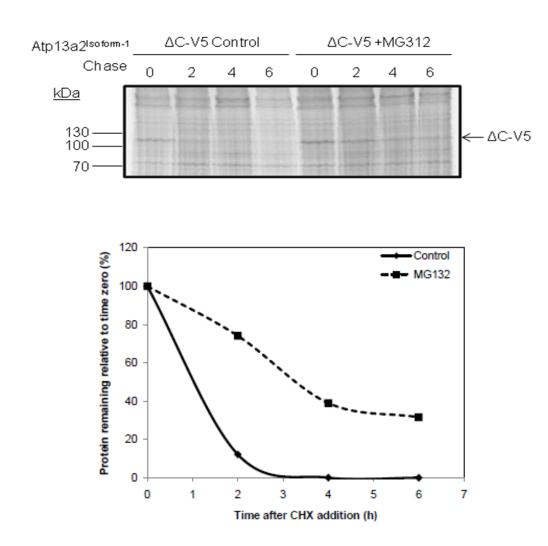


Figure S6. Pulse-chase analysis of Atp13a2^{Isoform-1}. (A) HeLa cells were transfected with V5tagged Δ C Atp13a2^{Isoform-1}. Twenty hours after transfection cells were pulse-labeled with [³⁵S]methionine and chased with nonradioactive medium containing MG132 or DMSO (vehicle control) for 0-6 hours. The Δ C protein was immunoprecipitated from the lysates using an anti-Atp13a2 antibody and separated by SDS-PAGE. Radioactivity of the Δ C-V5 band was measured by phosphoimage analysis. (B) Graph representing the radioactivity measured in (A). The estimated half-life of the Δ C protein increased from approximately 1 hour (Control) to 3.5 hours (MG132) which is similar to the results of our CHX-chase experiments. (C) HeLa cells stably expressing V5-tagged wild type Atp13a2^{Isoform-1} were subjected to the same conditions as described above for the Δ C mutant. Phosphoimage analysis of the immunoprecipitates showed the wild type protein to be relatively stable. Attempts to quantify the wild type band were unsuccessful due to the high background present.



Β



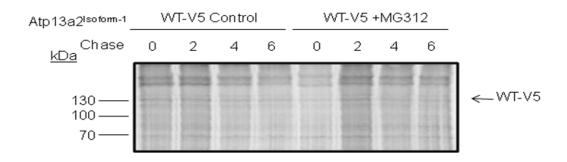


Figure S7. Wild type Atp13a2^{Isoform-1} Turnover. HeLa cells stably expressing V5-tagged wild type Atp13a2^{Isoform-1} were treated with cycloheximide and MG132 or DMSO vehicle for 12 and 24 hours. Lysates were immunoblotted for V5 and tubulin. The half-life of the protein was calculated to be in excess of twenty-four hours.

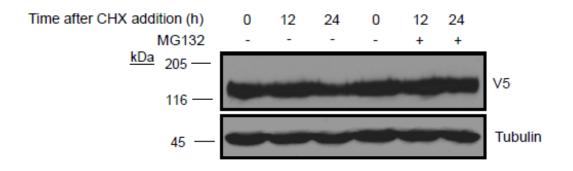


Figure S8. Expression of Atp13a2^{Isoform-3} and Quantification of Cell Death. HeLa cells were transfected with the indicated GFP constructs and cell death assays were performed as described previously for Atp13a2^{Isoform-1}. Graph is an average of three independent experiments and the data is shown as the mean ± standard deviation of the mean. The extent of cell death for the different Atp13a2^{Isoform-3} constructs was compared to that of the GFP control. Independent analysis was performed for the tunicamycin treatment and non-treatment. Expression of wild type or mutant Atp13a2^{Isoform-3} results in an significant increase in cell death compared to control GFP cells with or without tunicamycin **P* < 0.0005 ** P <0.005. By this analysis wild type Atp13a2^{Isoform-3} differs from Atp13a2^{Isoform-1} in that it increases cell death to the same extent as the Ex13 and Δ C mutants.

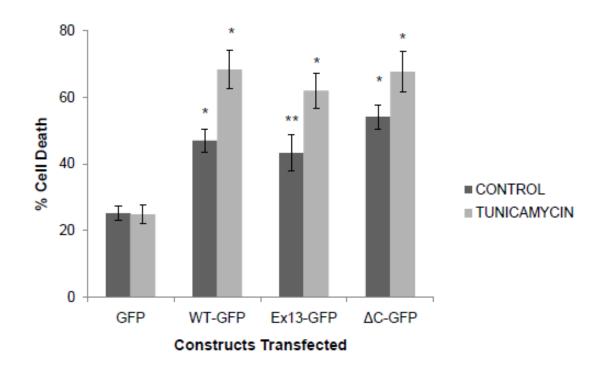


Figure S9. Expression of Atp13a2^{Isoform-1} and Quantification of BiP Levels. HeLa cells were transfected with GFP alone or with the indicated GFP-tagged Atp13a2^{Isoform-1} constructs. Twenty hours after transfection, the cells were treated with tunicamycin or DMSO vehicle control for 8 hours at a final concentration of 2 µg/ml. (A) Equal amount of lysates were separated by SDS-PAGE and immunoblotted for BiP, GFP, and Actin. (B) Quantification of BiP levels in cells that were either treated or not treated with tunicamycin all normalized to the cells transfected with GFP alone without tunicamycin treatment (average of three independent experiments). BiP levels in the cells transfected with GFP alone without tunicamycin-treated cells, except that the wild type did not show a significant increase compared to the mutants. However, we should note that the efficiency of transfection differed between the constructs, which could have underestimated the real differences. Data are shown as mean \pm standard deviation of the mean. (**P* < 0.05).



